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Experimental *Trypanosoma cruzi* cardiomyopathy in BALB/c mice: histochemical evidence of hypoxic changes in the myocardium

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Summary. Mice inoculated three times at intervals of 15 days with epimastigote forms of an 'avirulent' strain of *Trypanosoma cruzi* and challenged 30 days after the last inoculation with trypomastigote forms of the 'Colombia' strain of *T. cruzi* develop a cardiomyopathy very similar to that observed in the chronic phase of Chagas' disease in man. The most conspicuous histopathological finding in both human and experimental chagasic cardiomyopathy is focal myocardial necrosis and degeneration. Based on the nature of cell necrosis and degeneration, and the association of this lesion with intravascular platelet aggregation in the experimental model, we suggested that the microcirculation could be involved, via transient ischaemia, in the pathogenesis of chagasic cardiomyopathy. Additional support to this hypothesis is given by the results of the present study showing histochemical evidence of hypoxic changes in the myocardium of mice chronically infected with *T. cruzi*.

Keywords: *Trypanosoma cruzi*, cardiomyopathy, myocardial necrosis, hypoxic changes, mice

Previous work from our laboratory (Rossi *et al.* 1984) has shown that mice inoculated three times at intervals of 15 days with epimastigote forms of an 'avirulent' strain to *Trypanosoma cruzi* and challenged 30 days after the last inoculation with trypomastigote forms of the 'Colombia' strain of *T. cruzi* develop a cardiomyopathy very similar to that observed in the chronic phase of Chagas' disease in man. The cardiac syndrome is characterized grossly by cardiomegaly, with hypertrophy, dilatation of ventricular chambers, and thinning of the left ventricular apex, and microscopically by foci of myocytolytic necrosis and degeneration with an inflammatory exudate composed of mononuclear cells, with concurrent interstitial fibrosis and occasional myofibres containing pseudocysts. In addition aggregated platelets and occlusive thrombi were found in small myocardial vessels of infected mice but not in controls. It was assumed that these microcirculatory lesions may lead to localized tissue hypoxia and, consequently, to focal myocardial necrosis and degeneration; this could be important in the development of cardiomyopathy. This paper reports histochemical evidence of hypoxic changes in the myocardium of mice chronically infected with *T. cruzi*.

Materials and methods

Male BALB/c mice aged 5 to 6 weeks were inoculated three times at intervals of 15 days with 1×10^7 epimastigote forms of the 'PF'

strain of *Trypanosoma cruzi* (Menezes & Albuquerque 1970) and challenged 30 days after the last inoculation with 2×10^4 trypomastigote forms of the 'Colombia' strain of *T. cruzi* (Federici *et al.* 1964). Control mice were injected with saline instead of 'PF' and 'Colombia' strains of *T. cruzi*.

The animals were housed (5–6 per cage) in polypropylene cages, maintained under controlled conditions, and given laboratory chow and water *ad libitum*. Between 80 and 100 days after the challenge the mice were killed under light ether anesthesia by exsanguination from the abdominal aorta. The thoracic cavity was opened exposing the still-beating heart. The hearts of nine mice from the infected group and of six mice from the controls were fixed entire in neutral 10% formalin for histological study. All hearts were sectioned coronally from apex to base into two separate pieces including both ventricles and atria. After paraffin embedding the blocks were sectioned at 6 μm, stained with haematoxylin–eosin and a specific hypoxia staining method (Vértesi & Szentiványi 1981), and examined by light microscopy. The extent of hypoxic areas were evaluated in five or six serial sections from each block. The basic steps of hypoxia staining are: removal of paraffin, hydration with graded alcohols, staining with solution A (ferric-ammonium sulphate, 3.0 g; crystal violet, 0.2 g; distilled water, 97.0 g) for 2 min, rinsing in distilled water, staining with solution B (haematoxylin, 0.5 g; 96% alcohol, 5.0 g; mercury (II) oxide, 0.5 g; distilled water, 95.0 g) for 2 min, rinsing in distilled water, differentiation with solution A for 1–2 min, repeatedly washing in distilled water for 10 min, rinsing in distilled water, dehydration through graded alcohols, clearing in xylene, and mounting in Canada balsam. This staining method produces a dark-blue coloration in the hypoxic areas. The validity of the method was confirmed by local ligation of the coronary of vessels: a positive reaction develops within a few minutes of ligation of the coronary arteries in rats (Vértesi & Szentiványi 1981).

Results

The control mice showed no cardiac lesion.

The morphology of the hearts of infected mice has been reported elsewhere (Rossi *et al.* 1984). In particular, sections from hearts of experimental animals had small areas of myocardial necrosis and degenerative changes associated with a marked inflammatory cell infiltrate consisting of mononuclear cells (predominantly macrophages and a few lymphocytes) and fibrosis. The degenerative changes consisted of foci of myolysis with loss of linear arrangement of myofibrils with cross-striations, hyalinization of the cytoplasm, lighter staining zones within the cytoplasm, and loss of myocardial nuclear staining. Parasitism of the cardiac fibres was minimal, and the pathological changes in the myocardium were not correlated with the presence of parasites (Fig. 1 *a*, *b*). With the specific hypoxia staining method, small hypoxic areas appeared irregular and dark-blue, more or less sharply demarcated from the surrounding lightly stained myocardium (Fig. 1*c*, *d*).

Fig. 2 is a schematic representation of coronal sections through mice hearts infected with *T. cruzi*, with and without apical aneurysm, showing the extent of the hypoxic

Fig. 1. Myocardium of infected mice. (*a*) Foci of myocytolytic necrosis (arrow heads) and myofibres showing degenerative changes characterized by hyalinization of the cytoplasm (small arrows) and lighter staining zones in the cytoplasm (small arrow heads). Mononuclear inflammatory infiltrate. H & E. × 147. (*b*) Higher magnification showing zones of myocytolytic necrosis (arrow heads). Dense inflammatory infiltrate. Hyalinization of myofibres (small arrows). H & E. × 368. (*c*) Area of the apex of the left ventricle. Hypoxic areas of the myocardium are darkly stained, and are more or less sharply demarcated from the surrounding lightly stained muscle cells. Specific hypoxia staining method. × 58. (*d*) Higher magnification showing the darkly stained hypoxic myocardium surrounded by the light stained muscle. Specific hypoxia staining method. × 147.

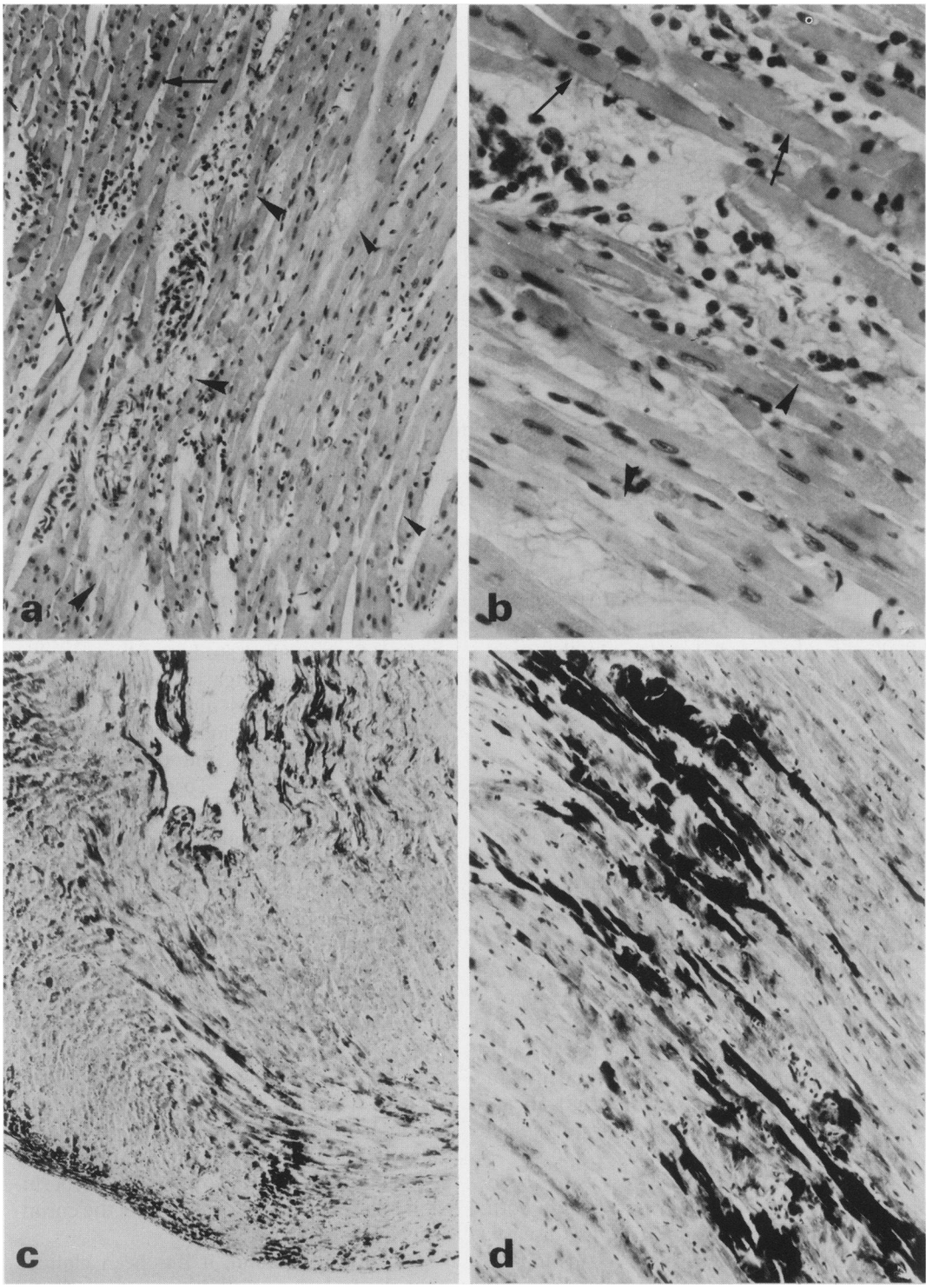

Fig. 1. Caption opposite

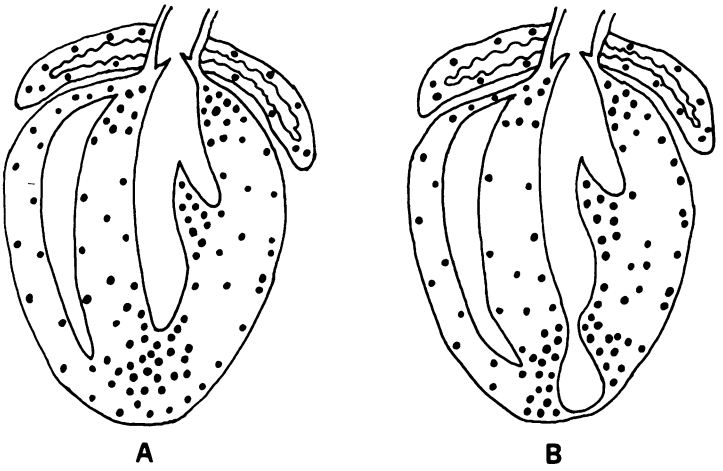

Fig. 2. Schematic representation of coronal sections through mice hearts infected with *T. cruzi* without (*a*) and with (*b*) apical aneurysm, showing the extent of the hypoxic areas. These areas are scattered throughout the ventricular and atrial myocardium, but are more numerous in the subendocardial and subepicardial regions in the apex, papillary muscles and base of the ventricles.

areas. These areas were scattered throughout the ventricular and atrial myocardium, but occurred particularly in the subendocardial and subepicardial regions in the apex, papillary muscles and base of the ventricles.

Discussion

These results provide direct histochemical evidence for the presence of myocardial hypoxia in the chronic experimental cardiomyopathy of mice infected with *T. cruzi*. Taking into account the findings of this study and previous observations showing intravascular platelet aggregation in this experimental model (Rossi *et al.* 1984), it is likely that myocardial hypoxia is a cause, via microcirculatory lesions, of the focal myocellular necrosis and degeneration observed in mice chronically infected with *T. cruzi*, and also an important factor in the development of cardiomyopathy and, in particular, of apical aneurysm of the left ventricle.

The involvement of small vessels has been reported previously by a few authors in both human and experimental Chagas' disease. Necrotizing arteritis was observed in biopsies from the cardia in cases of human chagasic megaesophagus (Brito & Vasconcelos 1959) and in the digestive tract of mice experimentally infected with *T. cruzi* (Okumura *et al.* 1960). Comparative studies of human chagasic and non-chagasic hearts showed marked irregularities and constrictions of intramyocardial arteriolar vessels in extensive myocytolysis in the chagasic group (Torres 1958, 1960). Torres (1960) suggested that the diffuse myocytolysis found in chagasic cardiomyopathy resulted from metabolic changes of the myocells due to circulatory disturbances of low intensity or short duration. More recently, a histotopographic study comparing the microcirculatory system after injection of an opaque medium in human chagasic hearts and in controls, showed chagasic focal decapillarization; it was suggested this could be the cause of the focal myocytolysis (Jörg 1974). In fact, a similar form of myocardial focal lesion has been observed both experimentally (Jennings & Ganote 1974) and clinically (Bulkley & Hutchins 1977) when reperfusion follows transient occlusion of the coronary circulation.

On the other hand, it has been claimed that the foci of myocytolytic necrosis found

in chagasic cardiomyopathy are very similar to the lesions produced by catecholamines, and it is possible they could result from an increased sympathetic tonus due to destruction of the cardiac parasympathetic innervation in Chagas' disease (Köberle 1968; Oliveira 1976). The pathomechanism by which catecholamines lead to myocardial necrosis appears to be due either to relative coronary insufficiency caused by a direct stimulation of the metabolism of the myofibre or to a local vasospasm reducing the oxygen supply to the cell (Johansson *et al.* 1981).

More recently, microvascular abnormalities have been assumed in the pathogenesis of unrelated human and animal models of heart diseases characterized by focal myocardial necrosis and subsequent fibrosis. It is well known that a microangiopathy occurs in both human and experimental diabetes, and this has been implicated, at least in part, in the pathogenesis of diabetic cardiomyopathy. Factor *et al.* (1981) showed extensive focal areas of myocardial fibrosis in hypertensive diabetic rats in association with microcirculatory lesions characterized by constrictions, tortuosities and true microaneurysm. Similar findings were described previously in human diabetic hearts (Factor *et al.* 1980). Vertési & Szentiványi (1981) demonstrated the development of hypoxic areas in the myocardium of streptozotocin-induced diabetic rats, which could be prevented by given a beta-blocking agent. Factor *et al.* (1982) studied the hereditary cardiomyopathy of the Syrian hamster using silicone rubber perfusion of the arterial tree and pretreatment of young animals with verapamil, a coronary vasodilator calcium-channel blocking agent; he suggested that the foci of myocardial necrosis in the cardiomyopathic hamster resulted from microvascular spasm due to vascular hyperreactivity to vaso-active substances.

Further investigations using microvascular perfusion both *post mortem* and in experimental models are warranted, and could lead to the eventual prevention of myocytolytic necrosis with anti-platelet and coronary vasodilator drugs.

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